Identification of a GTP-Binding Protein alpha Subunit that Lacks an Apparent ADP-Ribosylation Site for Pertussis Toxin

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Identification of a GTP-binding protein α subunit that lacks an apparent ADP-ribosylation site for pertussis toxin

(signal transduction/human retina/gene family)

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ABSTRACT **Recent molecular cloning of cDNA for the α subunit of bovine transducin (a guanine nucleotide-binding regulatory protein, or G protein) has revealed the presence of two retinal-specific transducins, called T_r and T_c, which are expressed in rod or cone photoreceptor cells. In a further study of G-protein diversity and signal transduction in the retina, we have identified a G-protein α subunit, which we refer to as $G_z\alpha$, by isolating a human retinal cDNA clone that cross-hybridizes at reduced stringency with bovine T_r α-subunit cDNA. The deduced amino acid sequence of $G_z\alpha$ is 41–67% identical with those of other known G-protein α subunits. However, the 355-residue $G_z\alpha$ lacks a consensus site for ADP-ribosylation by pertussis toxin, and its amino acid sequence varies within a number of regions that are strongly conserved among all of the other G-protein α subunits. We suggest that $G_z\alpha$, which appears to be highly expressed in neural tissues, represents a member of a subfamily of G proteins that mediate signal transduction in pertussis toxin-insensitive systems.**

Guanine nucleotide-binding regulatory proteins (G proteins) are a family of receptor-coupled signal-transducing proteins that regulate a variety of second-messenger systems and ion channels. Members of this family of structurally and functionally homologous proteins are involved in the regulation of retinal cyclic GMP phosphodiesterase, adenylate cyclase, phospholipase C, phospholipase A_2, and subsets of K^+ and Ca^{2+} channels (1–3). G proteins are generally found as heterotrimers composed of diverse α, β, and γ subunits. Diversity in the structure of G proteins has been shown by the identification of at least seven α-subunit cDNA clones that are encoded by separate genes (reviewed in ref. 1). Distinct β subunits, called β_1 and β_2, also exist (4, 5), as well as at least two forms of γ subunits (6–8).

G proteins are often referred to by the type of α subunit found in the holoprotein. The α subunit can bind GDP or GTP depending upon the state of its associated receptor and undergoes reversible association with βγ complexes. Different subfamilies of α subunits have been found and classified based upon their similarities in amino acid sequence. Some of the Gα proteins, such as the α subunits of the retinal rod and cone transducins, $T_r\alpha$ and $T_c\alpha$, are present only in a single cell type (9), while others, such as those of G_s (which stimulates adenylate cyclase), the G_i group (G_{i-1}, G_{i-2}, and G_{i-3}, which are structurally related G proteins), and G_o (whose function is unknown), appear to be widely expressed. The amino acid sequence of each Gα protein is extremely well conserved through evolution. For example, the bovine, rodent, and human $G_s\alpha$ proteins have at least 98% amino acid sequence identity. All of the Gα proteins have characteristically conserved amino acid sequences, and it has been argued that these mediate Gα-specific function. Furthermore, all of the Gα proteins found thus far have sites that act as substrate for ADP-ribosylation by either cholera toxin, pertussis toxin, or both. However, there are reports of signal-transduction processes that appear to require GTP but are not affected by toxins (10–14). We sought to further explore the diversity of Gα proteins, and in this paper we report the characterization of a cDNA clone† that represents a G-protein α subunit distinct from $G_s\alpha$, $G_{i(1-3)}\alpha$, $G_o\alpha$, $T_c\alpha$, and $T_r\alpha$.

METHODS

Screening of the Human Retinal cDNA Library. Clone λα161 was isolated from a human retinal λgt10 cDNA library that was constructed by Jeremy Nathans (Johns Hopkins University School of Medicine, Baltimore). The library was screened with a bovine $T_r\alpha$ cDNA plasmid probe (pML7) that contained a 2.19-kilobase (kb) *Nco* I cDNA fragment including the entire protein-coding region of bovine $T_r\alpha$. Hybridization was performed at 42°C in 20% (vol/vol) formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM NaH_2PO_4, pH 7.0/2× concentrated Denhardt's solution/0.1% $NaDodSO_4$ containing 50 μg of salmon sperm DNA per ml. (Denhardt's solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone.) The filters were washed at 37°C in 0.3 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ for low stringency and at 50°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$ for high stringency. Selected clones that hybridized to $T_r\alpha$ cDNA under conditions of low stringency only were rescreened by hybridization to a ^{32}P-labeled synthetic oligodeoxynucleotide probe (TαZ) and cDNA probes for rat $G_s\alpha$, $G_{i-1}\alpha$, $G_{i-2}\alpha$, $G_{i-3}\alpha$, and $G_o\alpha$ (kindly provided by David Jones and Randall Reed, Johns Hopkins University School of Medicine, Baltimore) and bovine $T_c\alpha$. The nucleotide sequence of TαZ, which was based on the amino acid sequence of $G_o\alpha$ (residues 42–53; Fig. 2), is 5′ d(CCICTTAGICCITTTTCGTGITAICAITTTGTTTAC) 3′ [with deoxyinosine (I) substituted at some of the third-base codon positions].

Hybridization Analysis of mRNA and Genomic DNA. Mouse spleen DNA was obtained from the Mouse DNA Resource (The Jackson Laboratory). DNA from human placenta was prepared as described (15). Hybridization to genomic DNA blots was carried out at 65°C in 0.9 M NaCl/0.09 M sodium citrate/5× Denhardt's solution/1% $NaDodSO_4$/10% dextran

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Abbreviations: G protein, guanine nucleotide-binding regulatory protein; T_r, rod transducin; T_c, cone transducin; G_s and G_i, G proteins that stimulate and inhibit adenylate cyclase; G_{i-1}, G_{i-2}, and G_{i-3}, structurally related G proteins; G_o, a G protein of undefined function; Gα, G-protein α subunit.

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†This sequence is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03260).

sulfate. Final washing conditions were 65°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$.

Total poly(A)$^+$ RNA samples from quick-frozen bovine tissues were prepared, size-fractionated by 1.0% agarose/2.2 M formaldehyde gel electrophoresis, and transferred directly to a nylon membrane as previously described (4). The filter was hybridized to nick-translated probe at 42°C in 50% formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM NaH_2PO_4/2× Denhardt's solution/0.1% $NaDodSO_4$ containing 50 μg of salmon sperm DNA per ml. Washing was done at 50°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$.

RESULTS

Bovine $T_r\alpha$ cDNA was hybridized at reduced stringency to about 42,000 plaques from a human retinal λgt10 cDNA library, and 309 preliminary positive clones were detected. About 230 of the positive clones remained hybridized to the $T_r\alpha$ cDNA probe after washing under conditions of high stringency. The other candidate positive clones, which hybridized only under conditions of lower stringency, were analyzed further. DNA was prepared from 16 of these cDNA clones, blotted onto nylon membranes, and hybridized to a labeled synthetic oligonucleotide, TαZ. The sequence of TαZ (see *Methods*) was derived from a strongly conserved amino acid sequence within all Gα proteins. It hybridized strongly to four of the cDNA clones, three of which were classified as G_o by hybridization to a rat G_o cDNA probe and by partial nucleotide sequencing. However, the fourth TαZ-hybridizing clone, λα161, failed to show strong hybridization to any of the cDNA probes for known α subunits (T_c, G_s, $G_{i\text{-}1}$, $G_{i\text{-}2}$, $G_{i\text{-}3}$, or G_o). λα161 was analyzed further by complete nucleotide sequencing of the 2.7-kb cDNA insert (Fig. 1).

Translation of the cDNA sequence in one open reading frame gave a 355-residue protein sequence (M_r = 40,920) that showed 66–67% amino acid sequence identity with $G_{i\text{-}1}\alpha$, $G_{i\text{-}2}\alpha$, and $G_{i\text{-}3}\alpha$; 60% identity with $G_o\alpha$; 55–57% identity with $T_r\alpha$ and $T_c\alpha$; and about 41% sequence identity with $G_s\alpha$. By analogy with the homologous Gα proteins the initiation codon was assumed to be the ATG at nucleotide position 1 (Fig. 1). Two consensus polyadenylylation signals (AATAAA) are present in the cDNA sequence at nucleotide positions 2156 and 2649. The second polyadenylylation site is closely followed by a 17-nucleotide-long poly(A) tract at the 3′ terminus. The protein encoded by the cDNA is clearly homologous to members of the Gα gene family, and we refer to it as $G_z\alpha$.

A comparison of the amino acid sequence of $G_z\alpha$ with those of other Gα proteins, $G_s\alpha$, $G_{i\text{-}2}\alpha$, $G_o\alpha$, and $T_c\alpha$, each representative of a particular class of Gα proteins, is shown

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-12  GAG ACA AGG ACC ATG GGA TGT CGG CAA AGC TCA GAG GAA AAA GAA GCA GCC CGG CGG TCC CGG AGA ATT GAC CGC CAC CTG CGC TCA GAG
                     Met Gly Cys Arg Gln Ser Ser Glu Glu Lys Glu Ala Ala Arg Arg Ser Arg Arg Ile Asp Arg His Leu Arg Ser Glu
                                                      10                                                  20
 79  AGC CAG CGG CAA CGC CGC GAA ATC AAG CTG CTC CTG CTG GGC ACC AGC AAC TCA GGC AAG AGC ACC ATC GTC AAA CAG ATG AAG ATC ATC
     Ser Gln Arg Gln Arg Arg Glu Ile Lys Leu Leu Leu Leu Gly Thr Ser Asn Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile
                    30                                                  40                                                  50
169  CAC AGC GGC GGC TTC AAC CTG GAG GCC TGC AAG GAG TAC AAG CCC CTC ATC ATC TAC AAT GCC ATC GAC TCG CTG ACC CGC ATC ATC CGG
     His Ser Gly Gly Phe Asn Leu Glu Ala Cys Lys Glu Tyr Lys Pro Leu Ile Ile Tyr Asn Ala Ile Asp Ser Leu Thr Arg Ile Ile Arg
                    60                                                  70                                                  80
259  GCC CTG GCC GCC CTC AGG ATC GAC TTC CAC AAC CCC GAC CGC GCC TAC GAC GCT GTG CAG CTC TTT GCG CTG ACG GGC CCC GCT GAG AGC
     Ala Leu Ala Ala Leu Arg Ile Asp Phe His Asn Pro Asp Arg Ala Tyr Asp Ala Val Gln Leu Phe Ala Leu Thr Gly Pro Ala Glu Ser
                    90                                                  100                                                 110
349  AAG GGC GAG ATC ACA CCC GAG CTG CTG GGT GTC ATG CGA CGG CTC TGG GCC GAC CCA GGG GCA CAG GCC TGC TTC AGC CGC TCC AGC GAG
     Lys Gly Glu Ile Thr Pro Glu Leu Leu Gly Val Met Arg Arg Leu Trp Ala Asp Pro Gly Ala Gln Ala Cys Phe Ser Arg Ser Ser Glu
                    120                                                 130                                                 140
439  TAC CAC CTG GAG GAC AAC GCG GCC TAC TAC CTG AAC GAC CTG GAG CGC ATC GCC GCA GCT GAC TAT ATC CCC ACT GTC GAG GAC ATC CTG
     Tyr His Leu Glu Asp Asn Ala Ala Tyr Tyr Leu Asn Asp Leu Glu Arg Ile Ala Ala Ala Asp Tyr Ile Pro Thr Val Glu Asp Ile Leu
                    150                                                 160                                                 170
529  CGC TCC CGG GAC ATG ACC ACG GGC ATT GTG GAG AAC AAG TTC ACC TTC AAG GAG CTC ACC TTC AAG ATG GTG GAC GTG GGG GGG CAG AGG
     Arg Ser Arg Asp Met Thr Thr Gly Ile Val Glu Asn Lys Phe Thr Phe Lys Glu Leu Thr Phe Lys Met Val Asp Val Gly Gly Gln Arg
                    180                                                 190                                                 200
619  TCA GAG CGC AAA AAG TGG ATC CAC TGC TTC GAG GGC GTC ACA GCC ATC ATC TTC TGT GTG GAG CTC AGC GGC TAC GAC CTG AAA CTC TAC
     Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Gly Val Thr Ala Ile Ile Phe Cys Val Glu Leu Ser Gly Tyr Asp Leu Lys Leu Tyr
                    210                                                 220                                                 230
709  GAG GAT AAC CAG ACA AGT CGG ATG GCA GAG AGC TTG CGC CTC TTT GAC TCC ATC TGC AAC AAC AAC TGG TTC ATC AAC ACC TCA CTC ATC
     Glu Asp Asn Gln Thr Ser Arg Met Ala Glu Ser Leu Arg Leu Phe Asp Ser Ile Cys Asn Asn Asn Trp Phe Ile Asn Thr Ser Leu Ile
                    240                                                 250                                                 260
799  CTC TTC CTG AAC AAG AAG GAC CTG CTG GCA GAG AAG ATC CGC CGC ATC CCG CTC ACC ATC TGC TTT CCC GAG TAC AAG GGC CAG AAC ACG
     Leu Phe Leu Asn Lys Lys Asp Leu Leu Ala Glu Lys Ile Arg Arg Ile Pro Leu Thr Ile Cys Phe Pro Glu Tyr Lys Gly Gln Asn Thr
                    270                                                 280                                                 290
889  TAC GAG GAG GCC GCT GTC TAC ATC CAG CGG CAG TTT GAA GAC CTG AAC CGC AAC AAG GAG ACC AAG GAG ATC TAC TCC CAC TTC ACC TGC
     Tyr Glu Glu Ala Ala Val Tyr Ile Gln Arg Gln Phe Glu Asp Leu Asn Arg Asn Lys Glu Thr Lys Glu Ile Tyr Ser His Phe Thr Cys
                    300                                                 310                                                 320
979  GCC ACC GAC ACC AGT AAC ATC CAG TTT GTC TTC GAC GCG GTG ACA GAC GTC ATC ATA CAG AAC AAT CTC AAG TAC ATT GGC CTT TGC TGA
     Ala Thr Asp Thr Ser Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Gln Asn Asn Leu Lys Tyr Ile Gly Leu Cys END
                    330                                                 340                                                 350
1069  GG AGCTGGGCCC  GGGGCGCCTG  CCTATGGTGA  AACCCACGGG  GTGTCATGCC  CCAACGCGTG  CTAGAGAGGC  CCAATCCAGG  GGCAGAAAAC AGGGGGCCTA
1171  AAGAATGTCC CCCACCCCTT GGCCTCTGCC TCCTTGGCCC CACATTTCTG CAAACATAAA TATTTACGGA TAGATTGCTA GGTAGATAGA CACACACACA TGCACACACA
1281  CACATCTGGA GATGGCAAAA TCCTCTAAAA TGTCGAGGTC TCTTGAAGAC TTGAGAAGCT GTCACAAGGT CACTACAAGC CCAACCTGCC CCTTCACTTT GCCTTCCTGA
1391  GTTGGCCCCA CTCCACTTGG GGGTCTGCAT TGGATTGTTA GGGATAGGCA GCAGGGCTGA GGCAAGGTAG GCCAACTGCA CCCCTGTCAC CTGGAGGAGG GCCGGCTCGC
1501  TGCCCGAGCT CTGGCCTAGG GACCTTGCCG CTGACCAAGA GGGAGGACCA GTGCAGGGTC TGTGCACCTT CCCTGCTGGC CTGCACACAG CTGCTCAGCA CCATTTCATT
1611  CTGGACCTGG GACCTTAGGA GCCGGGTGAC AGCACTAACC AGACCTCCAG CCACTCACAG CTCTTTTTAA AAAACAGCTT CAAAATATGC AGCAAAAACC AATACAACAA
1721  AACGAGTGGC ACGATTTATT TCAAACTAGG CCAGCTGGGA TTCCAGCTTT TCTTCTACTA GTCTGATGTT TTATAAATCA AAACCTGGTT TTCCTTCTCT GGCATTTTTT
1831  TTTGTTTTTT GTTTTTTGGT TTTTTTTTT TTTTTGGCCA AATCTCGTGG TGTTTCGCAG AAAAAAATCC AGAAAATTTC AAATGCAGTT GAGTATTCTT TTTTAAATGC
1941  AGATTTTCAA AACATATTTT TTTTCAGGTG GTCTTTTTTG TGTCTGGCTT GCTGAGTGTA AAAGTTGTTA TCTGGACGAT CTGTCTCTCT GCTCCAAAGA AATTTTGGAG
2051  TGAGTGGCAG TCCTGCGCCA GCCTCGCGGG ACACGTGTTG TACATAAGCC TCTGCAGTGT CCTCTTGTTA ATGGTGGGGT TTTCTGCTTT GTTTTTATTT AAGAAAATAA
2161  ACACGACATA TTTAAAGAAG GTTCTTTCAC CTGGGAGCAA ATGAACAATA GCTAAGTGTC TTGGTATTTA AAGAGTAAAT TATTTGTGGC TTTGCTGAGT GAAGGAAGGG
2271  GAGCAAGGGG TGGTGCCCCT GGTCCCAGCA TGCCCCGCGC CTGAGACTGG CTGGAAATGC TCTGACTCCT GTGAAGGCAC AGCCAGCGTT GTGGCCTGAG GGAGGCCCTG
2381  CTGGGACCCT GATCTGGGCC TTCCTGTCCC AGGGCCTATG GGCAACTGCG TTGAAAGGAC GTTCGCCAAG GGCCGTGTGT AAATACGAAC TGCGCCATGG AGAGGAGAGG
2491  CACTGCCGGA GCCCTTGCCA GATCTCCCTC CCTCTCTCTG TGCAGTAGCT GTGTGTCCGA GGTCAGTGTG CGGAATCACA GCCAAGGACG TGAAGAGATG TACGGGGGAA
2601  AGAGAAGCTG GGGATTGGAT GAAAGTCAAA GGTTGTCTAC TTTAAGAAAA TAAAATACCC TGAATGGAAA AAAAAAAAAA AAAA
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FIG. 1. Nucleotide and amino acid sequence derived from a retinal cDNA clone, λα161, encoding human $G_z\alpha$ subunit. Numbering of the nucleotide sequence is relative to the ATG initiation codon and is on the left. The amino acid sequence is numbered below the corresponding residues. Both strands of the cDNA were sequenced by the dideoxy chain-termination method, using synthetic 21-nucleotide-long primers (16).

in Fig. 2. Amino acid sequence differences that are unique to $G_z\alpha$ are scattered throughout the sequence, and some of these changes are also found within stretches of highly conserved amino acid sequence.

The dispersed distribution of the amino acid sequence differences between $G_z\alpha$ and the other G-protein α subunits suggests that $G_z\alpha$ is not derived by differential splicing from a known $G\alpha$ gene. To test this notion further we hybridized radiolabeled $G_z\alpha$ cDNA to blots of human placental and mouse spleen DNA cut with various restriction enzymes (Fig. 3). The pattern of restriction fragments hybridizing with the $G_z\alpha$ probe was compared with that detected with cDNA probes for all the other $G\alpha$ proteins derived from rat or human tissues (unpublished data). The $G_z\alpha$ pattern was clearly distinct, with few if any restriction fragments in common with the patterns obtained with the other probes. These results suggest that there is a unique gene that encodes $G_z\alpha$ both in humans and in mice.

Since $G\alpha$ protein sequences and mRNA distribution appear to be well-conserved between humans and a number of other mammals, we used bovine RNA from different tissues to study the expression of $G_z\alpha$. Hybridization of a $G_z\alpha$ cDNA probe to bovine poly(A)$^+$ RNA revealed $G_z\alpha$ expression in a number of tissues (Fig. 4). A major mRNA transcript about 3.0 kb long and minor transcripts of 2.5 and 2.2 kb were detected. The abundance of $G_z\alpha$ mRNA was highest in neural tissues (i.e., retina and brain) and relatively low or absent in other tissues. The 3.0-kb transcript predominated in retina, brain, adrenal gland, kidney, and liver; however, it was not detected in testis, lung, and spleen. A faint 2.5-kb band was also present in retina RNA. The different mRNA species may represent different gene products that result from tissue-specific alternative splicing or the selection of alternative polyadenylylation or transcriptional initiation sites. The results also do not exclude the possibility that the individual transcripts derive from separate but highly conserved genes.

DISCUSSION

On the basis of its unique primary structure, the size and pattern of its expressed mRNA, and its distinct array of hybridizing genomic fragments, the $G_z\alpha$ cDNA represents a novel $G\alpha$ protein. $G_z\alpha$ is most homologous to the G_i-like α subunits, having 66–67% identity in amino acid sequence to $G_{i\text{-}1}$, $G_{i\text{-}2}$, and $G_{i\text{-}3}$ subunits. However, with respect to one another, the G_i-like α proteins are 85–94% identical in amino acid sequence and appear to form a separate subfamily or

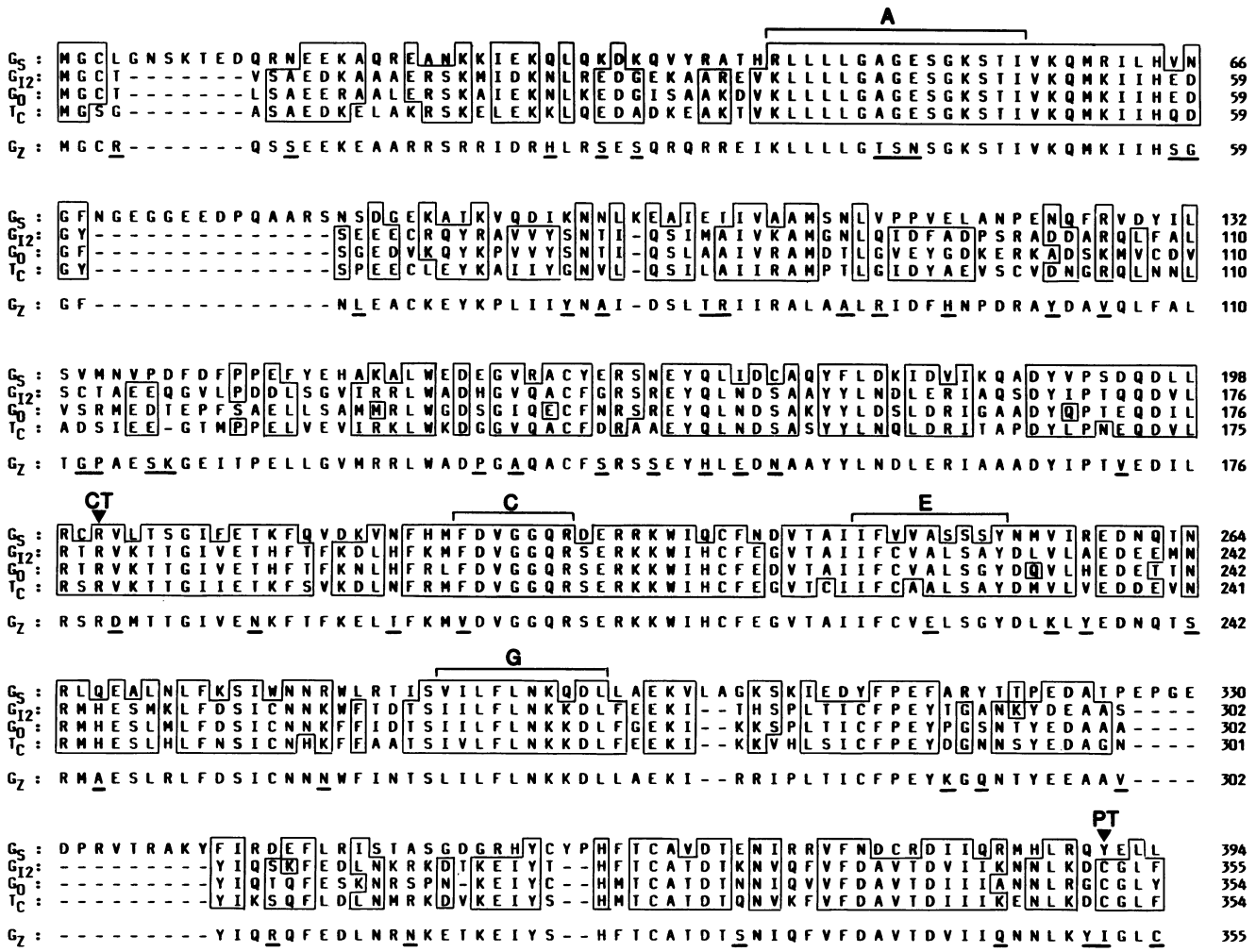

FIG. 2. Alignment of the amino acid sequences for the deduced G_z-protein α subunit and α subunits for human G_s (17), human $G_{i\text{-}2}$ (18), rat G_o (19), and bovine T_c (20). The homologous proteins were aligned with respect to $G_z\alpha$ by using the FASTP algorithm developed by Lipman and Pearson (21). Amino acid residues are represented by standard one-letter symbols. Dashes indicate gaps inserted to optimize homology. Three or more identical or conserved residues in a column are boxed. The amino acid residues in $G_z\alpha$ that are not homologous to any of the corresponding residues at the same position in the other $G\alpha$ proteins are underlined. Postulated guanine nucleotide-binding and -hydrolysis domains A, C, E, and G (22, 23) are indicated above the sequences. Arrowheads point to sites of ADP-ribosylation by pertussis toxin (PT) and cholera toxin (CT) analogous to the sites identified in $T_r\alpha$ (24, 25).

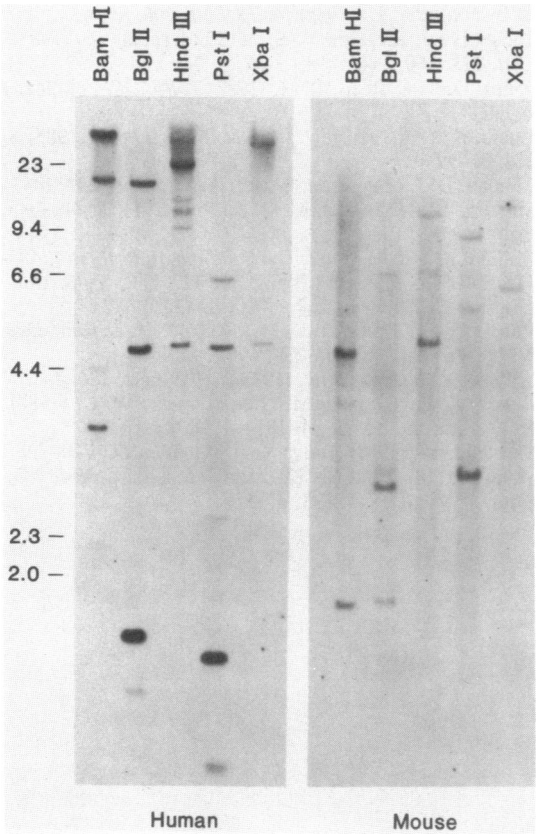

FIG. 3. Hybridization analysis of human and mouse genomic DNA. Samples (8 μg) of DNA from human placenta or mouse spleen were cut with the indicated restriction enzymes and analyzed by blot hybridization to the 2.7-kb $G_z\alpha$ cDNA probe. These same filters and other similarly prepared blots were hybridized to other α-subunit cDNA probes for G_s, G_{i-1}, G_{i-2}, G_{i-3}, G_o, T_r, and T_c. No overlapping patterns of restriction fragments were found (unpublished data). *Hin*dIII fragments of phage λ DNA were used as size markers (lengths in kb at left).

class of α subunits. $T_r\alpha$ and $T_c\alpha$, which are about 80% identical in amino acid sequence, form another group of α chains. $G_z\alpha$ is as divergent from the $G_i\alpha$ group as are $G_o\alpha$, $T_r\alpha$, and $T_c\alpha$, and is therefore best categorized within a separate class of Gα proteins.

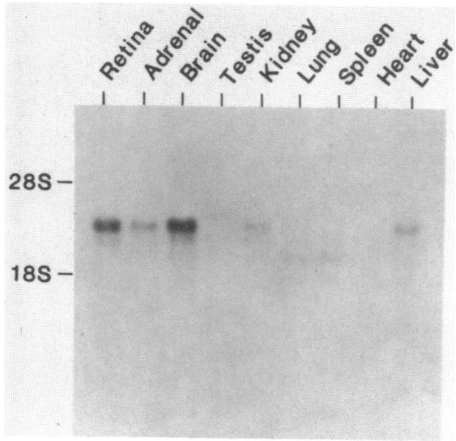

FIG. 4. Blot hybridization analysis of $G_z\alpha$ mRNA transcripts in bovine tissues. Each lane contained 1 μg (retina) or 2 μg (all other tissues) of bovine poly(A)$^+$ RNA. The complete 2.7-kb $G_z\alpha$ cDNA was labeled by nick-translation and used as hybridization probe. RNA lengths were determined relative to bovine 28S and 18S rRNA size markers.

In all known Gα proteins, several regions or domains have been identified as ones that may be involved in guanine nucleotide binding or hydrolysis (1, 22). These regions are homologous in amino acid sequence to the putative GDP-binding regions, designated the A, C, E, and G regions in the sequence of translation elongation factor EF-Tu and $p21^{ras}$ proteins (23). Like other Gα proteins, $G_z\alpha$ shows highly conserved amino acid sequences in these regions (Fig. 2). However, there are a few notable variations in the $G_z\alpha$ sequence. In particular, in the A region $G_z\alpha$ is different in sequence from other Gα proteins, all of which contain a perfectly conserved 18-amino acid stretch. This conserved region includes an amino acid sequence motif, Gly-$(Xaa)_4$-Gly-Lys (specifically Gly-Thr-Ser-Asn-Ser-Gly-Lys in $G_z\alpha$ and Gly-Ala-Gly-Glu-Ser-Gly-Lys in all other Gα proteins), that is found in many mononucleotide-binding proteins, including $p21^{ras}$ and elongation factors EF-Tu and EF-1α (26, 27). Mutations in the corresponding sequence motif in $p21^{ras}$ have been correlated with decreased GTPase activity, enhanced oncogenic capacity, and tumor progression (28). The mononucleotide-binding and -hydrolysis properties of $G_z\alpha$ remain to be determined.

In some cases sequence differences in $G_z\alpha$ may be interpreted in terms of potential effects on its properties. For example, $G_z\alpha$ does not contain a typical amino acid sequence for ADP-ribosylation by pertussis toxin near the carboxyl terminus. Gα proteins that are pertussis toxin substrates, including T_r, G_o and G_i-like α subunits, terminate with the common sequence Cys-Gly-Leu-(Phe or Tyr) in which the conserved cysteine is the ADP-ribose acceptor site (24). This common sequence and the conserved cysteine are absent in $G_z\alpha$ as well as in $G_s\alpha$, which is known not to be modified by pertussis toxin. The ability of $G_z\alpha$ to be ADP-ribosylated by cholera toxin might also be compromised due to an amino acid difference in $G_z\alpha$ adjacent to Arg-179, which corresponds to the site of ADP-ribosylation by cholera toxin in $T_r\alpha$ (25).

Since $G_z\alpha$ does not contain a typical pertussis toxin modification site, it may mediate transduction in signaling systems that are not blocked by the toxin. There are several examples of receptor-mediated stimulation of phospholipase C that appear to involve G proteins but are insensitive to the effects of pertussis toxin, including, for example, the action of vasopressin, muscarinic agonists, and thyrotropin-releasing hormone in various cell types (10–14). Thus, one possible function of G_z may be to control phospholipase C activity in specific cells. The identification and characterization of $G_z\alpha$ by molecular cloning provides an important means to purify the $G_z\alpha$ gene product and study its distribution, biochemistry, and function.

Note Added in Proof. Kaziro and his colleagues (29) have isolated a human genomic clone and characterized a rat brain cDNA clone that correspond to the human $G_z\alpha$ cDNA reported in this paper.

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